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Cross-species amplification of *Lolium* microsatellites in *Poa* ssp

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Abstract

Cross-species amplification of 47 *Lolium* ssp. microsatellite primers were evaluated across eight *Poa* species or subspecies. Of the 47 evaluated *Lolium* simple sequence repeat (SSR) primer pairs examined, 18 generated one or more amplification products. Of these, only two resulted in the identification of *Poa* ssp. microsatellite motifs, of which only one was complementary to the microsatellite motif identified in *Lolium*. Though few *Poa* ssp. microsatellite regions were identified, several of the amplification products were polymorphic within and across the *Poa* ssp. and could be utilized as markers in *Poa* ssp. intergeneric hybrid studies. Results of the research suggest the use of *Lolium* microsatellite derived primers to identify complementary SSR regions in *Poa* is not an effective approach for the development of microsatellite markers in *Poa*.

Introduction

Representatives of the *Poa* genus have been classified into over 300 taxonomically identified species that are commonly utilized as durable and persistent forage and turf grasses (Huff 1992). *Poa* is an extremely diverse genus, generally considered to be allogamous, exhibiting both sexual and apomictic, and monoecious and dioecious forms. In addition, the large amount of *Poa* genetic diversity provides a considerably useful germplasm resource for an inter-specific hybrid evaluation program (Kindiger 2004a,b). However, few molecular markers are available for any *Poa* species and this places restrictions on a research program focused on marker-assisted-introgression, genotyping and phylogenetic studies. As a consequence, it would be useful to develop an assortment of informative molecular markers that would have application in such studies.

Successful amplification of cross-species SSR marker analysis is known to be limited among distantly related species and is most successful when applied to members representing the same genus or presumably closely related (Whitton *et al*. 1997; Westman and Kresovich 1998; Kaplinsky *et al*. 2002). Several studies have indicated this can be a successful approach for developing microsatellite markers in plants (Agrawal *et al*. 1999; Gao *et al*. 2003). *Poa* is a member of the tribe Poaceae (Yaneshita *et al*. 1993) and has been suggested to be allied with the *Lolium–Festuca* species complex (Kubik *et al*. 1999). Limited SSR cross-species amplification studies

utilizing *Lolium perenne* SSR primer pairs have been successfully evaluated in *Poa pratensis* (Kentucky Bluegrass) with a reported success level of 38% to 46% (Kubik *et al*. 1999; Jones *et al*. 2001) and that DNA sequence comparisons of the amplification products suggest the potential for identifying orthologous loci between *Lolium perenne* and *Poa pratensis* (Jones *et al*. 2001).

Though the genetic relationship of *Poa* to the *Lolium–Festuca* genetic complex has been implied, few *Lolium* microsatellite primer pairs have actually been evaluated in *Poa* and their potential application across a wide range of *Poa* ssp. has not been investigated. As a consequence, representatives of the *Poa* genus that are commonly found or utilized as forage in rangeland, pasture and native grasslands, were evaluated with a series of *L. perenne and L. multiflorum* SSR primer pairs to determine if they can identify a set of informative and useful SSR markers for an array of *Poa* ssp.

Materials and methods

Plant materials

This study included five indigenous North American species; *P. arachnifera* Torr., *P. arida* Vasey, *P. alpina* L., *P. secunda* Presl., *P. secunda* Canbyi, two Australian species, *P. rodwayi* Vick., *P. labillardieri* Steud., one Argentine species, *P. ligularis* Nees ex. Steud. and one European species, *P. pratensis* L.

Poa arachnifera was obtained from the germplasm collection at the USDA-ARS, Grazinglands Research Laboratory, El Reno, OK, USA. *Poa rodwayi*, *P. labillardieri* and *P. ligularis* were provided by the Tasmanian Institute of Agricultural Research, Mt Pleasant Laboratories, Kings Meadows, Tasmania. *Poa alpina* cv. AEC Glacier was obtained from Alberta Research Council, Vegreville, Alberta, Canada. *P. secunda* cv. Sherman (PI578850) and *P. arida* cv. Reno (PI578806) were obtained from the USDA Western Regional Plant Introduction Stn., Pullman, WA, USA *P. secunda* cv. Canby was provided by Round Butte Seed Growers, Culver, OR, USA. *P. pratensis* experimental variety SS22 (forage type), cv. Baron (turf type), cv. Barthetia (turf type) and cv. Barrister (turf type) were provided by Barenburg Seeds, Albany, OR, USA.

The mode of reproduction in the selected *Poa* species ranged from sexual to facultative apomixis. *Poa arachnifera* and *P. ligularis* support a purely sexual form of reproduction while *P. secunda*, *P. alpina*, *P. arida*, *P. labillardieri*, *P. rodwayi* and *P. pratensis* support a facultative aposporous form of apomictic reproduction. With the exception of *P. pratensis*, three or more individuals were evaluated from each species, irrespective of their mode of reproduction. For the *Poa pratensis* evaluations, one individual representing the four apomictic commercial cultivars was evaluated. Since the commercial *P. pratensis* cultivars are near obligate apomictics, using more than one individual from each cultivar would rarely identify any polymorphism. As a consequence, four commercial apomictic cultivars were selected for the study.

To examine the potential value and expression of any potential markers, a series of *Poa* interspecific hybrids was included in the study. Each of the *Poa* ssp. interspecific hybrids was generated at the USDA-ARS, Grazinglands Research Laboratory, El Reno, OK, USA by crossing a female *P. arachnifera* individual with pollen obtained from a particular individual representing a particular *Poa* ssp. (Kindiger 2004a). The *Lolium multiflorum* cultivar Mara was utilized as the control for polymerase chain reaction (PCR) and for sequence comparisons to the *Poa* ssp. amplification products.

Primers, polymerase chain reaction (PCR) amplification, electrophoresis and sequence comparison

DNA extractions were performed using fresh leaf material and following the manufacturer's instructions in the Epicentre Leaf DNA extraction kit. The *Lolium perenne* and *L. multiflorum* SSR primer pairs were obtained from the references provided in Table 1a and were synthesized by Sigma-Genosys. A touchdown PCR protocol was utilized in the evaluations to reduce the frequency of possible mispriming events and dimer–primer products (Don *et al*. 1991). Each 25.5 μL PCR amplification reaction consisted of 12.5 μL Sigma RedTaq PCR Reaction Mix; 10 μL ddH20, 1 μL primer pair and 2 μL (10 ng) of genomic DNA. PCR amplifications were generated in a MWG Primus-96 thermal cycler. Prior to this study, a series of experimental PCR amplification programs was evaluated to identify a particular protocol that would provide optimum results across a wide range of materials. The use of a single PCR protocol also simplified the utilization of these markers, negating the need to have a particular optimized PCR protocol for each primer pair. As a consequence, a touchdown PCR protocol was identified and used for this study. The PCR protocol is as follows: 3 min 95°C 1 cycle; 1 min 95°C, 1 min 72°C, 1 min 60°C 1 cycle (repeat and reduce annealing temperature by 1°C until it reaches 56°C); 1 min 95°C, 1 min 72°C, 1 min 55°C 24 cycles; 4 min 72°C, hold at 4°C. A 100–1008 bp size standard (Promega Co., Madison, WI, USA) was included on each gel and the size of the amplification product was visually estimated. All PCR products were evaluated on a 4% Amresco-SFR agarose gel with a run time of approximately 1.5 h. The Amresco-SFR agarose is a high quality agarose that has proven effective in detecting microsatellite variation in genetic studies of maize (Davis *et al*. 1999). As a consequence, the utilization of a high quality agarose to investigate potential genetic marker variability in this study was appropriate. Gels were stained with EtBr and examined with a UV transluminator.

Two criteria were used to identify potential SSR regions in and across the examined *Poa* ssp. Since SSR polymorphisms

Table 1 (a) *Lolium multiflorum and L. perenne* SSR primers and primer sequences evaluated across the eight *Poa* ssp

SSR Primer ID	Forward	Reverse
(A)		
NGLRI LM 4–9F	ATCGGACACTGGTTCCGCAT	TTGTTGTTGCCGGCTTCGTA
NGLRI LM 12–10F	AATTCAACCATGCAAGGAGG	GCGGCATTAGCAACAAGC
NGLRI LM 3–11B	GGCAAGTGCAAATCCATTGC	CCAAATTGGGCTTCCCAAGA
NGLRI LM 3–4C	CTTCTATGGTTGACCTGCTGCG	TAAGGGCATGAGTGCGGTCA
NGLRI LM 2–5A	TGCACATCACACACATACTTGAGC	TTTGCTTGCTAGTGTTCGTTGTG
NGLRI LM 7–1D	CTAGACCACACCGAGATGACCG	AAGGCCACTCAATCAAAGAGATTG
NGLRI LM 2–1B	CTTTATTGCACTTTACTTGCCTTGC	CGATGTTCCACGTCAGGTGG
NGLRI LM 3–4E	CTCAAAATGAGCCCGCCTTG	CCGACGATGATGACGCATTC
NGLRI LM 3–1F	CCCTCCCACGTGAAAAGTGG	GTTCGGCACACCAACTCACG
NGLRI LM 1–11G	AGTTGTTGTTGCCTCTCGAGAACC	TACCGATCTGAATCGATCCTCCAT
NGLRI LM 1–11A	GCTGTTCTGCTCGGATCCTG	CGAGTTATCTAGCTATCTCTTTAGTCAGGG
NGLRI LM 1–1B	GCACCTATTCATGGCTCCGG	GCTTTGCCGGTTATGGCTCC
NGLRI LM 4–10C	CAGTGTTCCAAACTCCGCGA	CAGTCGGAGTGCTGATGATCG
NGLRI LM 3–4F	CAGATGGGCAGTTGCCACTG	GTATTGTACACACAAGCATATTGGCG
(B)		
LPSSRH01A02	AAAGACCGCATACGAAGT	AACCAAAGCCTCAAGACA
LPSSRH01A07	TGGAGGGCTCGTGGAGAAGT	CGGTTCCCACGCCTTGC
LPSSRH01A10	GAGGCACCGGCCATGGAG	AGGACGAGCCACTCACTTG
LPSSRH01D09	CAAGTGCCACCATAGATACAA	CGTGAAGATCACTATAAACACGA
LPSSRH01E10	CGCAGCTTAATTTAGTC	GCTTTGAGTATGTAAAGTT
LPSSRH01F02	TCTGTGGGTCCTTCTGGAT	TCGGGTGATGATGTTGACTT
LPSSRH01H06	ATTGACTGGCTTCCGTGTT	CGCGATTGCAGATTCTTG
LPSSRH02D11	TGGAATAACGATGAAAAG	CATCACGAATTAACAAGAG
LPSSRK01A03	GGACGAACTGCCGACACA	CGGGCATGGTGAGAAGGA
LPSSRK01A11	CGGCCACCCTTGATAGAG	TCGTCAAGGATCCGGAGA
(C)		
M2-148	GCAACTTCTATCGAGTTG	GAGGCTCGATCTTCACGGA
M12-52	CTACAATGCATTCGTGCA	TAGAGGCACCCGCGCCCT
(D)		
LP8	TGACTTCTCTCGATCCT	ATGTGACTACAAAACCA
LP20	ACCGCTGTGCTAAATCTG	ATGCGCTGCTGTCTGCCCT
LP165	CCATCACCTCCACTAT	AGCTCGCAGTCTGTTG
LP194	GCGTAAGAGAGAGGGCGAT	ACGTATGTCCAACAGGT
LP204	GAGCTTCTCTCGATCCT	AGTGGATGTGACTACA
M16B	TGCTGTGGCTCTTGTGAC	AGCCGAGGCTCAGCTCGA
M144	CAGAAGGAGGTCGTCGA	CTGAAACCTAGGCTATCTGAG
M844	TAGCTTTCTATGCAAAGCT	CACTTCACTTTTCTTGCA
M4136	AGAGACCATCACCAAGCC	TCTGGAAGATTTCCTTG
M4213	CACCTCCCGCTGCATGGCATGT	TACAACGACATGTCAAGGT
M10138	TAGAGGATCAGTTGCATC	TAGTTCCGAGTTAGCTGA
M15185	GGTCTGGTAGACATGCCTAC	TACCAGCACAGGCAGGTTC
PRE	CATTCATCCACGTTAGAC	GTTAGGTTCGTCTGCAT
PRG	GCCGAGTGTCATCAAGGT	CTTTTTCGCCTTCGTA
PR3	GTATAGTACCCATTCCGT	GCCGCCCTGCCATGCTG
PR8	AGGGTTCGTCTGCATTC	GCCGTCGCACCCCTG
PR10	CTTCTAATCCCTCGCCT	TGCCGAGTGTCATCAAGGT
PR14	CCTTTTCGCCTTCGTA	CACCAACATTGCCGAGTG
PR24	TGCTGTGATGCTGAATG	GTATAGTACCCATTCCGTTGTC
PR25	AGGGTTCGTCTGCATTC	CCTGCATACATTCATCCA
PR37	TCTGCATTCGTTGTCTCACTG	GAGCCGTCGCACCCCTG

Primer sequences were obtained from the following sources: (A) M. Fujimori, Nat. Institute Livestock and Grassland Sci., Tochigi, Japan; (B) Jones *et al*. 2001 (C) Kubik *et al*. 1999; (D) Kubik, C. *et al*. 2001.

Table 1 (b) *Lolium* ssp. Simple sequence repeat primer pairs that were identified as providing useful markers across the *Poa* ssp. The molecular provided weights are approximate. Identical molecular weights across *Poa* ssp. do not indicate the lack of polymorphisms since in some instances, null alleles were identified

Primer ID	Motif	Par	Pal	Pa	Pp	PsS	PsC	Plig	Plab	Prod	Lolium
LM3–1F	$(GT)_{12}$	NA	550	1200, 1000	NA	NA	NA	NA	NA	NA	380
LM3–4C	$(CA)_{11}TGCT(CA)_8$	800	750	100, 900	100, 350	450	NA	550	NA	800	290
LM3–11B	$(GT)_{14}$	170	170	170	170, 390	NA	NA	170	NA	170	170
LM12–10F	$(AC)_{2d}(AC)_3AG(AC)_3$	NA	500	1000	NA	NA	NA	1000	1000	1000	180
LPSSRH01A07	$(GT)_9$	450, 190	NA	NA	450	450	450	NA	NA	450	400
LPSSRH01D09	$(AG)_8$	800	NA	1000	NA	NA	NA	800	800	800, 280	280
LPSSRH01F02	$(TCGC)_6$	1000, 800	250	NA	1000, 450	NA	900	1000	1000	1000	150
LPSSRH01H06	$(CA)_9$	170	170	170	170	170	170	170	170	180	180
LPSSRK01A11	$(CA)_{21}$	NA	NA	NA	NA	NA	NA	NA	1000	1000	200
M2-148	$(GT)_9(GA)_9$	1000	1000	750	800	1000, 700	700	750	750	750	180
M12–52d	$(GA)_9$	400	400	400	400	400	NA	400	400	400	150
M16B	$(GA)_3G(GA)_{18}GG(GA)_7$	180	180	180	180	180	180	180	180	180	180
M144	$(CT)_{16}$	1000	1000	1000	1000	1000	1000	1000	1000	1000	NA
M1038	$(CA)_{10}$ $AA(CA)_3$	450	420	NA	400, 180	500	500	400, 380	180	180	120
PRG	$(CA)_{13}$	900	900	900	900	900	900	900	900	900	180
PR3	$(CA)_{22}$	500, 350	380	380	550	700, 380	700	NA	NA	800	NA
PR8	$(GT)_{14}GC(GT)_{28}$	700	210	NA	800	700	700	NA	NA	NA	NA
PR10	$(GT)_2GG(GT)_{11}$	350	500	250	500	250	250	250	250	250	NA

Lolium = *Lolium multiflorum* cv. Mara; Pa = *P. arachnifera*; Pal = *P. alpina*; Par = *P. arida*; Plab = *P. labillardieri*; *Plig* = *P. ligularis*; Pp = *P. pratensis*; Prod = *P. rodwayi*; PsC = *P. secunda* cv. Canby; PsS = *P. secunda* cv. Sherman. NA, no amplification product generated.

are a product of short, repetitive base-pair motifs, it is anticipated that if complementary SSR regions exist between *Lolium* and *Poa*, the amplification products would exhibit similar molecular weight size to the *Lolium* check sequence (e.g. Figure 1a). Generally, the *Lolium* SSR would range in size from 100 to 300 bp. Though size similarity between the *Lolium* and *Poa* amplification products are not an absolute criterion for suggesting sequence similarity, it does provide an initial screen for identifying potentially complementary amplification products. The second selection criterion relied on the generation and observation of a typical SSR electrophoretic band profile that consists of only one to a few amplification products of similar molecular weight size. In instances where one or two bands are observed, these products were considered potential sites of complementary *Lolium–Poa* or *Poa–Poa* microsatellite regions. In instances where the *Lolium* check failed to amplify, any *Poa* products in the 100–300 bp size range were considered potential SSR if the amplification products were consistently generated within and across the *Poa* species. Only *Poa* amplification products within this size range were subjected to cloning, sequencing and microsatellite motif evaluation.

In instances where several large and small amplification products were generated and a complex banding profile was observed, such products were not sequenced or evaluated. Polymerase chain reactions yielding complicated banding profiles are considered to be the result of either mispriming events or the amplification of multiple or duplicate sites within the polyploid *Poa* ssp. genome. As a consequence, PCR reactions giving complex banding profiles were not considered potentially useful candidates for the identification of potentially useful *Poa* ssp. SSR markers. These criteria were universally applied toward the identification of potential *Poa* ssp. microsatellites generated by the *Lolium* SSR primer pairs.

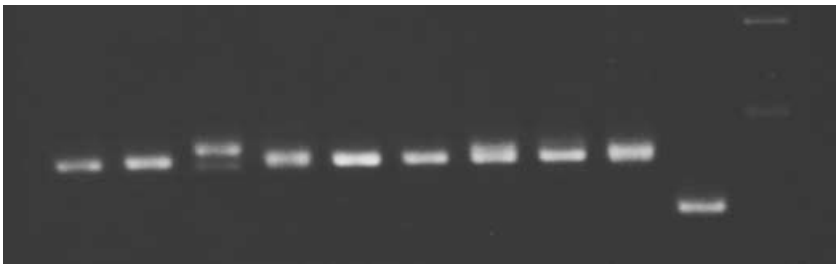
When potentially complementary *Lolium–Poa* ssp. or *Poa–Poa* amplification products were considered for sequencing, the PCR product from a single individual was cloned into the pCR 2.1 vector comprising the Invitrogen TA cloning kit, following the manufacture's instructions. Polymerase chain reactions using *Lolium* SSR primer pairs that did not have their amplification products sequenced are identified by a 'NA' designation in Table 1b. Single pass DNA sequencing was performed by Northwoods DNA, Becidia, MN, USA. Sequence comparisons are made using the DNA multiple alignment program ClustalW (Thompson *et al.* 1994).

Results and discussion

Amplification products

Poa represents a complex of interrelated polyploid species, each possessing the capacity for genome duplications, homoeologous loci, and distorted marker segregations with no regard to an individual's particular ploidy (e.g. 2x,

(a)



(b)

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Prod      CTTNCATTTCTTTTNAAAATTATTATCAT-----CAANAAAACCGCGA----CAAA--CA 49
Plabil    CTTNCATTTCTTT-GAAGATTATTGTCAG-----CAANAACCGTGAAT----CAAGGGCA 50
Pligul    --------CTTTNGAAAAATTATTNTCAATCAAACAAAAACCCGCGTA----CTAAAACA 48
Pprat2    ---TGANATNCCNCNAAAATTATTATCAT-----CAATAAAACCGCGA----TGAAA-CA 47
Psec      --CTGTATTGCTTNGAAAATTATTNNCNT-----CAACAAAACCGCGA----CCAA--CA 47
Parida    -CATANATTNCNCTGAAA-TTATTCC-AT-----CAACAAAACCGCGA----CCAA--CA 46
Palpina   ---ANANATNCCCTGAAGATTATTGTCAG-----CAANAAAACCGTGAAAAATCAAGGCA 52
Parach    ----CAGTATNCCCTGAAATTATTATCAG-----CAANAAAACCGCGA----TCAA--CA 45
Lolium    ---CAGTATTGCCTCGAAGTTATTGTCAG-----CAACAAAACCATGCA---TGAA--CA 47
                        *  *****          *** **                *   **

Prod      TCAG-GCACAAT-ACA-ATTCAACTGG--CTG-ACTGTCCA-ATGCCCACAGTACCGCAA 102
Plabil    TAGG-CCACAAT-ACA-ATT-AACTGG--CTG-ACTGTCCA-ATGCCCACAGGTCCGCAA 102
Pligul    TCTA-G-ACAAT-ACA-ATCAAACTTGTGCTGTACTGCTCC-AAATGCCCACTCCCGCTA 103
Pprat2    TCAG-GCACAAT-ACA-ATTCAACTGG--CTG-ACTGTCCA-ATGCCCACAG-ACCGCAA 99
Psec      TCAG-GCACAAT-ACA-ATTCAACTGG--CTG-ACTGTCCA-ATGCCCACAG-ACCGCAA 99
Parida    TCAG-GCACAAT-ACA-ATTCAACTGG--CTG-ACTGTCCA-ATGCCCACAG-ACCGCAA 98
Palpina   TCAG-G-GCAAA-ATATATTAAACTGT--CTG-ACTGTCCA-ATGCCCACAG-ACCACAA 104
Parach    TCAG-GCACAAT-ACA-ATTCAACTGG--CTGTACTGTCCAGATGCCCACAG-ACCGCAA 99
Lolium    TCAGTACACAANCACAGATTCAAATGT-------CCAGNGAAGTATGCACA----CACAC 96
          *    ***  * *  **  ** *          *           * **     * *
Prod      CTGCACG-AAGAAGATGACNTGCACACACCTGTAACAAGAATCTG-CAATCGCGAATTTT 160
Plabil    CTGCACG-AAGAAGATGACT-GCACACACCTGTAACAAGAATCTG-CAAT-GCGAATTTC 158
Pligul    ACGTAAG-AA-AGTATCAATGACCACCTCGTAAACAAAGAATCTGGCTATCGGTAATCTC 161
Pprat2    CCGCACGTAAGAAGATGACACACACGCACCTGTAACAAGAATCTG-CAATCGCGAAGCTC 158
Psec      CTGCATG-AAGATGATGACATACACGCTATAG---CAAGAATCTG-CAATCGCGAAGCTC 154
Parida    CTGCANG-AAGATGATGACATACACGCTATAG---CAAGAATCTG-CAATCACGAAGATC 153
Palpina   CTGCACGAAGCAAGATGACACACGCACACGTA-ACCTGTGACANG--AAGCGGCAATCGC 161
Parach    CTGCACG-AAGAAGATGACACACACACACGTAAGNCAAGNATTAA-CNNTCGNGAGANNN 157
Lolium    ACACATGTGNCAAGAT--TCTGCNNACNCGGAAGCCAGTCAATATANNNNNNNTNNNNNN 154
                *   *  *       *   *            *
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Figure 1 (a) A comparison of amplification products generated from *Lolium* simple sequence repeat (SSR) primer pairs LPSSRH01H06. Lanes 1 to 11 are identified as follows: L1, *P. arachnifera* (female); L2, *P. arachnifera* (male); L3, *P. alpina* cv. AEC Glacier; L4, *P. arida*; L5, *P. labillardieri*; L6, *P. ligularis*; L7, *P. pratensis* cv. Baron; L8, *P. rodwayi*; L9, *P. secunda* cv. Sherman; L10, *Lolium multiflorum* cv. Mara; L11, Promega 100 bp molecular weight marker (200, 300 bp markers shown). (b) ClustalW (v1.83) sequence comparison of an approximate 160 bp orthologous locus among *Lolium*, various *Poa* species. Individual designations and approximate size of original polymerase chain reaction products are: Psec (*Poa secunda* cv. Sherman); Plabil (*P. labillardieri*); Parida (*P. arida*); Proad (*P. rodwayi*); Pprat2 (*P. pratensis* cv. Barthetia); Palpina (*P. alpina* cv. AEC Glacier); Parach (*P. arachnifera* male plant); *Lolium* (*L. multiflorum* cv. Mara); Pligul (*P. ligularis* female plant). DNA sequences are aligned in 5′(→)3′ orientation with numbered bp positions. Common sequence identities across all taxa or species are indicated with an asterisk (*). In this instance, the *Lolium* (CA)n SSR repeat for LPSSRH01H06 is located at the 88–98 bp positions. (c) ClustalW (v1.83) sequence alignment comparison scores of an approximate 160 bp cloned LPSSRA10H06 amplification products generated in *Lolium* and seven *Poa* species.

3x, 4x, 5x and 6x). This has been well documented in prior attempts to utilize amplified fragment length polymorphisms to generate first generation maps in *P. pratensis* and *P. arachnifera* (Albertini *et al*. 2003; Renganayaki *et al*. 2005). In this study, single and multiple amplification products exhibiting identical, similar or disparate sequences were obtained following amplification with a particular *Lolium* SSR primer pair. It is important to note that in the absence of mapping and progeny analysis, sequence similarity does not demonstrate allelism of these PCR products or allow one to infer it. As a consequence, the visualization of multiple bands having similar sequences should be considered variable homoeologous loci in keeping with terminology utilized in describing variability in allopolyploid species such as wheat and cotton. As such, the terminology describing the expression of the markers as dominant or co-dominant is technically reserved for mapped loci with known alleles. The author is aware that the absence of mapping data precludes any attempt to consider amplification products with a similar sequence as alleles, thus allowing them to have a true co-dominant designation. Though several of the amplification products co-expressed in an F1 heterozygote, a co-dominant designation cannot be assigned to these amplification products until mapping and extensive progeny testing data can be obtained.

Using the described PCR protocol, good amplification products were obtained with 28 primer pairs of which 18 revealed polymorphisms across the *Poa* ssp. (Table 1b). In five instances, the PCR amplification products exhibited identical molecular weights across the various *Poa*, suggesting a lack of variation at that locus. Nine *Lolium* SSR primer pairs yielded weak or non-specific products and 10 failed to generate an amplification product in any *Poa* ssp. The estimated molecular weights of the *Poa* ssp. PCR amplification products ranged from approximately 180–1200 bp (Table 1b). Few *Poa* ssp. amplification products were similar in size to the *Lolium* amplification product, or were within the 100–300 bp criteria that was used to suggest potentially complementary *Lolium–Poa* amplification products. Only *Lolium* SSR primer pairs LPSSRH01H06, LM3–11B and M16B generated amplification products similar in size to the *Lolium* check. Most *Poa* ssp. amplification products were substantially larger than the *Lolium* amplification product (Table 1b). Figures 1a, 2a and 3a illustrate the three instances where the *Lolium* and *Poa* ssp. amplification products were of similar size and met all the criteria for cloning and sequencing and ClustalW

(c)

SeqA	Name	SeqB	Name	%Identity
1	Lolium	2	Parach	61
1	Lolium	3	Psec	58
1	Lolium	4	Palpina	57
1	Lolium	5	Pligul	13
1	Lolium	6	Parida	49
1	Lolium	7	Prod	47
1	Lolium	8	Pprat2	53
1	Lolium	9	Plabil	48
2	Parach	3	Psec	71
2	Parach	4	Palpina	71
2	Parach	5	Pligul	57
2	Parach	6	Parida	74
2	Parach	7	Prod	71
2	Parach	8	Pprat2	77
2	Parach	9	Plabil	68
3	Psec	4	Palpina	65
3	Psec	5	Pligul	61
3	Psec	6	Parida	80
3	Psec	7	Prod	74
3	Psec	8	Pprat2	74
3	Psec	9	Plabil	68
4	Palpina	5	Pligul	53
4	Palpina	6	Parida	64
4	Palpina	7	Prod	72
4	Palpina	8	Pprat2	73
4	Palpina	9	Plabil	74
5	Pligul	6	Parida	59
5	Pligul	7	Prod	68
5	Pligul	8	Pprat2	68
5	Pligul	9	Plabil	66
6	Parida	7	Prod	72
6	Parida	8	Pprat2	76
6	Parida	9	Plabil	67
7	Prod	8	Pprat2	80
7	Prod	9	Plabil	87
8	Pprat2	9	Plabil	72

Figure 1 *Continued.*

comparison. In addition, a wide variation in the size of the *Poa* amplification products, independent of species (Table 1b) was also observed. In some instances, the size of the *Poa* ssp. amplification products was similar (Figures 1a,2a). In other instances, considerable size differences were observed across the *Poa* species. As such, it is suggested that the molecular weights provided be treated as a representation of the possible degree of band size variability that could be identified across the sexual and apomictic *Poa* species, cultivars, accessions etc. Simply stated, due to the large amount of variability in the *Poa* genus, the primer pairs that provided informative amplification products of a particular molecular weight size in the materials used in this study are likely to vary depending on the source of the *Poa* germplasm used in the analysis. Predictability regarding the size and number of amplification products generated by evaluating the *Lolium* ssp. SSR primer pairs across this diverse array of *Poa* species cannot be inferred by the data provided in Table 1b nor is it suggested by the author.

Utilizing the *Lolium* SSR primer pairs to identify potential SSR sites in the *Poa* ssp. resulted in the generation of both simple (Figure 1a) and complex electrophoretic patterns. Since potential complementary *Lolium*-to-*Poa* microsatellite regions were the focus of this study, *Lolium* SSR primer pairs that generated complex PCR profiles in the *Poa* evaluations were not pursued. In some instances, faint molecular weight bands were observed. Faint bands are believed to be PCR artifacts and were not reliably amplified in replicated PCR reactions. Overall, 18 primer pairs generated reproducible amplification products across the 9 *Poa* ssp. (Table 1b).

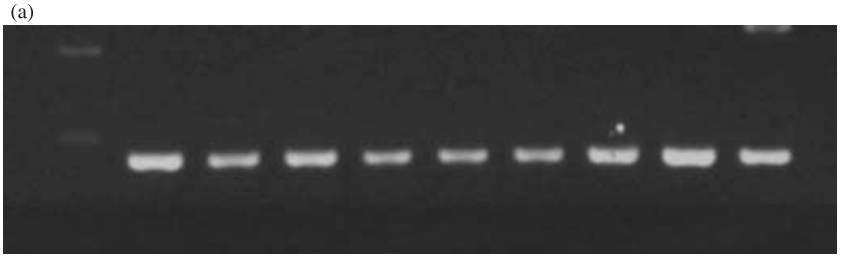
Sequence comparisons

Sequence data were obtained from amplification products generated by *Lolium* ssp. primer pairs LPSSRH01H06, LM3–11B and M16B. These were the only primer pairs to generate *Poa* amplification products that were similar in size to the *Lolium* amplification product and fit the previously described criteria for identifying potential complementary SSR regions.

For the *Lolium* primer pair LPSSRH01H06, an approximate 170 bp amplification product was generated (Figure 1a,b). In this example, the ClustalW sequence comparison illustrates that the approximate 170 bp amplification products exhibits a moderate degree of sequence similarity with *Lolium* and across several *Poa* ssp. (Figure 1c). However, the complementary *Lolium* SSR motif $(CA)_n$ was not identified in any of the *Poa* ssp. sequences.

In a second example utilizing data obtained from *Lolium* SSR primer pair LM3–11B, the ClustalW sequence comparisons of an approximate 170 bp amplification product suggests a high level of sequence similarity with the *Lolium* check and across the *Poa* species (Figure 2a,2b,c). As in the previous example, the LM3–11B *Lolium* microsatellite $(GT)_n$ motif was not identified in any of the examined *Poa* species. However, a *Poa* $(CT)_n$ repeat motif that closely aligned with the *Lolium* $(GT)_n$ motif was identified in the amplification products of *P. arida*, *P. rodwayi*, *P. arachnifera*, *P. pratensis*, *P. ligularis* and *P. alpina*. We can only speculate on the cause for these different motifs residing at similar positions. At this time, it is assumed that the presence of the identified *Poa* $(GT)_n$ repeat may simply be due to chance and does not allow us to infer some unrealized degree of *Lolium–Poa* genome similarity. In addition to the 170 bp amplification product, the LM3–11B primer pair also generated an approximate

(a)



(b)

```
Palpina   TTCCTTTATGGNGTCTGCAGGGGGCTTTTTCNGTGNTAACTGGNTTNNCACATCGG-ACA 59
Pligul    TTCCTTTATGGGGTCTTCNGGGGGCTTTTTCGGTGCTAACTGGGTTNTCACATCGGCACA 60
Parach    TTCCTTTATGGGGTCTGTAGGGGGCTTTTTCGGTGCTAACTGGGTTTTCACATCGGCACA 60
Parida    TTCCTTTATGGGGTCTGTNNGTGGGTTTT-CGGTGCTAACTGGGCTTTCACATCGGCACG 59
Lolium    -TTCTTTATGGGGTCTGTNGGGGGCTTTTTCGGTGCTAACTGGGTTTTCACATCGGCACA 59
Pprat2    -TTCTTTATGGGGTCTGTNNGGGGCTTTTTCGGTGCTAACTGGGTTTTCACATCGGCACA 59
Prod      TTCCTTTATGGGGTCTGTNNGGGGCTTTTTCGGTGCTAACTGGGTTTTCACATCGGCACA 60
            * ******** ****    * ** **** * *** *******   ********* **
Palpina   NATACTCTCTTCTAATTCCCCGTTAGNCTTCTCGAAAGATAACTTCTTANGAGACTTGAC 119
Pligul    GATACTCTCTTCTAATTCCCCGTTAGTCTTCTCGTAAGATAACTTCTNAGGAGACTTGAC 120
Parach    GATACTCTCTTCTAATTCCCCGTTAGTCTTCTCGTAAGATAACTTCTTAGGAGACTTGAC 120
Parida    GATGCTCTCTTCTAATTCCTCGTTAGTCTTCTCGTAAGATAGATTCTCAGGAGACTTGAC 119
Lolium    G-TGTGTGTGTGTAATTCCCCGTTAGTCTTCTCGTAAGATAACTTCTTAGGAGACTTGAC 118
Pprat2    GATACTCTCTTCTAATTCCCCGTTAGTCTTCTCGTAAGATAACTTCTTAGGAGACTTGAC 119
Prod      GATACTCTCTTCTAATTCCCCGTTAGTCTTCTCGTAAGATAACTTCTTAGGAGACTTGAC 120
            *     * ************** ******* ****** **** * **********
Palpina   TTTCTTGGGAAGCCCACTTTGGANNNNCNNNNNNNGNN---------- 157
Pligul    TTTCTTGGGAAGCCCACTTTGGANNNNNNNNNNNNNNNNNN------ 164
Parach    TTTCTTGGGAAGCCCACTTTGGANNNNNNNNNNNNNNNNNN---------- 160
Parida    TTTCTTGGGAAGCCCACTTTGGANNNNNNNNNNNNNNNNNNNNN--- 166
Lolium    TTTCTTGGGAAGCCCACTTTGGANNNNNNNNNNNNNNNNNA---------- 158
Pprat2    TTTCTTGGGAAGCCCACTTTGGNAGNCNCNCGNNCCNGACNNGNNGCN-- 167
Prod      TTTCTTGGGAAGCCCACTTTGGAATGTNGGGGAGNGGGGNNNTTTGANAC 170
          **********************
```

(c)

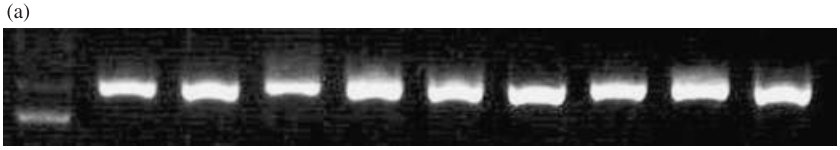
SeqA	Name	SeqB	Name	%Identity
1	Lolium	2	Parach	93
1	Lolium	3	Pprat2	97
1	Lolium	4	Palpina	85
1	Lolium	5	Pligul	91
1	Lolium	6	Parida	87
1	Lolium	7	Prod	84
2	Parach	3	Pprat	90
2	Parach	4	Palpina	91
2	Parach	5	Pligul	96
2	Parach	6	Parida	92
2	Parach	7	Prod	90
3	Pprat2	4	Palpina	83
3	Pprat2	5	Pligul	87
3	Pprat2	6	Parida	84
3	Pprat2	7	Prod	88
4	Palpina	5	Pligul	91
4	Palpina	6	Parida	84
4	Palpina	7	Prod	83
5	Pligul	6	Parida	90
5	Pligul	7	Prod	87
6	Parida	7	Prod	83

Figure 2 (a) Low molecular weight amplification products generated from *Lolium* simple sequence repeat (SSR) primer pair LM3–11B. Lanes 1 to 10 are identified as follows: L1, Promega 100 bp molecular weight marker (200–300 bp markers shown); L2, *P. arachnifera* (female); L3, *P. pratnesis* cv. Barthetia; L4, *P. pratensis* cv. S22; L5, *P. alpina* cv. AEC Glacier; L6, *P. arida*; L7, *P. labillardieri*; L8, *P. ligularis*; L9, *P. rodwayi*; L10, *Lolium multiflorum* cv. Mara. (b) ClustalW (v1.83) sequence comparison of an approximate 165 bp orthologous locus among *Lolium*, various *Poa* species. Individual designations and approximate size of original polymerase chain reaction products are: Parida (*P. arida*); Prod (*P. rodwayi*); *Lolium* (*L. multiflorum* cv. Mara); Pligul (*P. ligularis*); Parach (*P. arachnifera*, female plant); Pprat2 (*P. pratensis* cv. Barthetia); Palpina (*P. alpina* cv. AEC Glacier). *P. labillardieri* was not successfully sequenced and is not provided. DNA sequences are aligned in 5′(→)3′ orientation with numbered bp positions. Common sequence identities across all taxa or species are indicated with an asterisk (*). In this instance, the *Lolium* (GT)n SSR repeat for LM3–11B is located at the 60–72 bp position which closely aligns with an approximate (CT)5 repeat for the amplification products of *P. arida*, *P. rodwayi*; *P. arachnifera*, *P. pratensis*, *P. ligularis* and *P. alpina*. (c) ClustalW (v1.83) sequence alignment comparison scores of the approximate cloned 165 bp LM3–11B amplification products generated in *Lolium* and across six *Poa* species.

390 bp band that appeared to be specific only to *P. pratensis*. This amplification product was unrelated to the 170 bp product by sequence and was not generated in any of the other examined *Poa* species (data not shown).

In a third example, data for *Lolium* SSR primer pair M16B are provided. A ClustalW sequence comparison of the approximate 180 bp amplification products generated in the *Lolium* and the *Poa* ssp. suggests both similar and diverse sequence complementary (Figure 3a,3b,c). In this example, the *Poa* ssp. amplification products have a sequence similarity ranging from 2% to 88% with respect to the *Lolium* amplification product. *P. arachnifera*, *P. secunda*, *P. pratensis*, *P. rodwayi* and *P. arida* exhibit the highest level of sequence similarity to the *Lolium* amplification product; whereas *P. labillardier*i and *P. alpina* amplification products had little sequence similarity to any *Poa* or the *Lolium* check.

(a)



(b)

```
Psec      -----CTTCTGTGNACCCTTGTCC-----TGGCGGTGCGCTCTTGTG-ACCTGTTTGAGC 49
Parida    -----CTTCTGTGNACCCTTGTCC-----TGGCGGTGCGCTCTCCTG-ACCTGTTTGAGC 49
Lolium    -----NTTCTGTGNACCCTTGTCC-----TGGCGGTGCGCTCTTGTG-ACCTGTTTGAGC 49
Parach    TGTNNNNNCCTGNGACTGCTGTGT-TATTTGTTTGAGCAGCGTTCT----TTGTTTGAGC 55
Pligul    TGTNNNNNCCTGNGACTGCTGTGTCTATATTGTTGAGCAGCGTTCTG---TTGTTTGAGC 57
Pprat2    -TTNTNNNCTCTGNACTGCTGTGT--TTTTGTTTGAGCAGCGCTCT----TTGTTTGAGC 53
Prod      --------CTGTNGACTGCTGTGT-TATTTGTTTGAGCAGCGCTCTTTGATGTTTTGAGC 51
Palpina   --GANCTTTTGTGAGTTCTTTTCG------GTAGCTAAAATAATATGAATATTTCCGTGC 52
Plabil    CCNTCACTCTGTGTATAGCTAGTT------GGATGCCCGCTATTGTG--TACAGTGATAT 52
                                *                      *
Psec      GGCGTTCTTTGTTTTAGCGT---GAGAGAGAGAGAGAGA-----GA----TATGGCTTGGC 99
Parida    GGCGTTCTCTGTTTTAGCGAGAGAGAGAGAGAGAGAGA-----GA----TATGGCTTGGC 100
Lolium    GGC------TAGCGTAGAGA--GAGAGAGAGAGAGAGA-----GA----TATGGCTTGGC 92
Parach    ACCGAGTGTGGAGAGAGAGAGAG--GGAGATTGAGA-CACAGA----GATGGCTTG-C 107
Pligul    ACCGAG-----AGAGAGAGAGAG--GGAGATTGAGATCACAGATCGGAGTGGCTTG-C 109
Pprat2    ACCGAGTGTGGAGACAGAGGGAGAGTGAGAGATTGAGA-CACAGA----GATGGCTTG-C 107
Prod      ACCGAGTGTGGAGAGAGAGAGAGTGAGAGATTGAGA-CACTAT---GGATGTCTTG-G 106
Palpina   GACTAG----GTCTCGAAGCCGGCGCTATCATAACGGATTTAGGA-----CTCACTCTGT 103
Plabil    AGCGAGT----ACACGCATCAGCTGTGTCTGTCGCAGTTTGGGGTC---CATAGCCCACC 105
                *                      *                *
Psec      AACGGTTC----CTGCGACCACTACACTGCC--CT--CGAGCTAGAGCCTCGACTATCTC 151
Parida    AACGGTTCTTTACTGCGACCACTATACGGCCGGCTAGCGAGCTAGAGCCTCGACTATCTC 160
Lolium    AACGGTTC----CTGCGACCACTACACTGCC--CT--CGAGCTAGAGCCTCGACTATCTC 144
Parach    ACTGGCTC----GAGCGACGACTA-AGAGTGGGCT--CGAGCT-GAGCCTCGGCTANNNN 159
Pligul    AC--GCTC----GAGCGACGACTA-AGAGTGGGCT--CGAGCT-GAGCCTCGGCTGAGCC 159
Pprat2    ACTGGCTC----GATCTCCGA-TA-AGAGTGGGCT--CGAGCT-GAGCCTCGGCTACNNC 158
Prod      TCTCGCTC----C-CCTATCATTC-CCACT--ACT--CGTCCT-GACCCCCNNNNNTNTT 155
Palpina   ATTCCAACC---ATGTAGTCTTGATGTTTATTGTTTCCAAGAT--GTTTCTGGTTATCTA 158
Plabil    AGCTGATT----ACTCGCCTCGAATACGTCC------TGANNNNNNNNNNNNNNNNNNN 155
Psec      CCN-TCTGT-ATCNNNNN---------------- 167
Parida    CCN-NNNGTTATCNNNNN---------------- 177
Lolium    CCNCTCNNTTATNTNTATNNNTCNNNNNNN--- 174
Parach    NNNNNNNNNNTNNNNNNNNNNNNNNN------ 186
Pligul    TCGGCTACNNCT---------------------- 171
Pprat2    TANNNNNNNNNNNNNNNNNNNNNNNNNNNNN 191
Prod      NTATNNNNNNNNNNNN----------------- 171
Palpina   TCTATATTATATATATATAGAGACTAANTG--- 188
Plabil    NNNNNNNNNNNNNNNN----------------- 171
```

Figure 3 (a) The approximate 180 bp molecular weight amplification products generated from a *Lolium* simple sequence repeat primer pair M16B. Lanes 1 to 10 are identified as follows: L1, Promega 100 bp molecular weight marker (100–200 bp markers shown); L2, *Lolium multiflorum* cv. Mara; L3, *P. arachnifera* (male); L4, *P. pratnesis* cv. Barthetia; L5, *P. secunda* cv. Sherman; L6, *P. labillardieri*; L7, *P. alpina* cv. AEC Glacier; L8, *P. ligularis*; L9, *P. arida*; L10, *P. rodwayi*. (b) ClustalW (v1.83) sequence comparison of an approximate 180 bp orthologous locus among *Lolium*, various *Poa* species. Individual designations and approximate size of the original PCR products are: Psec (*Poa secunda* cv. Sherman); Parida (*P. arida*); *Lolium* (*L. multifolroum* cv. Mara); Parach (*P. arachnifera* male plant); Pligul (*P. ligularis*); Pprat2 (*P. pratensis* cv. Barthetia); Prod (*P. rodwayi*); Palpina (*P. alpina* cv. AEC Glacier); Plabil (*P. labillardieri*). DNA sequences are aligned in the 5′(→)3′ orientation with numbered bp positions. Common sequence identities across all taxa or species are indicated with an asterisk (*). In this instance, the approximate *Lolium* (GA)n SSR motif for M16B is located at the approximate 75–85 bp position of *P. secunda*, *P. arida*, *P. arachnifera*, *P. ligularis*, *P. pratensis*, *P. rodwayi*. The motif is absent in *P. alpina* and *P. labillardieri*. (c) ClustalW (v1.83) sequence alignment comparison scores of an approximate 180 bp clones M16B amplification product generated in *Lolium* and eight *Poa* species.

Evaluation of the sequences for the *Lolium* microsatellite motif $(GA)_3G(GA)_{18}GG(GA)_7$ revealed the presence of a similar $(GA)_n$ motif pattern in *P. arachnifera*, *P. secunda*, *P. pratensis*, *P. rodwayi* and *P. arida*. No similar pattern motif was identified in the sequences of the *P. labillardieri* and *P. alpina* amplification products.

All other amplification reactions using the *Lolium* SSR primer pairs across the *Poa* ssp. failed to generate an amplification product, generated many amplification products, or generated amplification products that greatly differed in size when compared to the *Lolium* SSR amplification product. Random selection, cloning and sequence comparisons of these products indicated that the *Lolium* and *Poa* amplification products were highly divergent, suggesting no potential regions of genomic complementation (data not shown).

Orthologous microsatellite sequences across *Lolium* and *Poa* ssp.

Previous research utilizing cross-species amplification of *Lolium* SSR primer pairs in *P. pratensis* suggested the potential for orthologous loci (Kubik *et al.* 1999; Jones *et al.* 2001). The varying degrees of sequence similarity exhibited between the amplification products of the *Lolium* SSR primer pairs LPSSRH01H06, LM3–11B and M16B across the *Poa* are in partial agreement with the suggestion of cross-species sequence conservation (Kubik *et al.* 1999). However, the failure of 44 *Lolium* SSR primers to generate similar amplification products across the same *Poa* ssp. implies a lesser degree of sequence conservation than may have been previously assumed.

(c)

SeqA	Name	SeqB	Name	% Identity
1	Lolium	2	Psec	88
1	Lolium	3	Parach	52
1	Lolium	4	Pprat2	63
1	Lolium	5	Parida	83
1	Lolium	6	Prod	49
1	Lolium	7	Pligul	48
1	Lolium	8	Plabil	2
1	Lolium	9	Palpina	8
2	Psec	3	Parach	64
2	Psec	4	Pprat2	59
2	Psec	5	Parida	94
2	Psec	6	Prod	51
2	Psec	7	Pligul	59
2	Psec	8	Plabil	2
2	Psec	9	Palpina	6
3	Parach	4	Pprat2	88
3	Parach	5	Parida	62
3	Parach	6	Prod	73
3	Parach	7	Pligul	85
3	Parach	8	Plabil	2
3	Parach	9	Palpina	2
4	Pprat2	5	Parida	47
4	Pprat2	6	Prod	76
4	Pprat2	7	Pligul	73
4	Pprat2	8	Plabil	2
4	Pprat2	9	Palpina	2
5	Parida	6	Prod	47
5	Parida	7	Pligul	32
5	Parida	8	Plabil	3
5	Parida	9	Palpina	3
6	Prod	7	Pligul	60
6	Prod	8	Plabil	3
6	Prod	9	Palpina	4
7	Pligul	8	Plabil	2
7	Pligul	9	Palpina	4
8	Plabil	9	Palpina	3

Figure 3 *Continued.*

However, irrespective of the high or low degree of sequence similarity identified by comparing *Lolium* and *Poa* ssp. amplification products, SSR motifs were identified in only two instances. These are the identification of a $(GT)_n$ repeat in an approximate 170 bp amplification product in *P. ligularis*, *P. arachnifera*, *P. arida*, *P. alpina*, *P. rodwayi* and *P. pratensis* that closely aligned with a *Lolium* $(CT)_n$ repeat using primer LM3–11B, and a $(GA)_n$ motif identified in some *Poa* ssp. using the *Lolium* primer M16B. This inability to identify no more than two *Lolium–Poa* microsatellite regions is in conflict with earlier data that suggests *Lolium* primers M16B, M4213, M15185 (Table 1a) could amplify *Poa pratensis* genome regions possessing complementary microsatellite regions to the *L. perenne* genome (Kubik *et al.* 1999). M16B did successfully identify a complementary microsatellite motif region between *L. perenne* and some *Poa* ssp., while M4213 and M15185 failed to generate any *Poa* amplification products. Since the *Poa* genus is very diverse, it is possible that the species and accessions used in this study are genetically distinct from the *P. pratensis* accessions evaluated in the previous studies (Kubik *et al.* 1999; Jones *et al.* 2001) and as such, it could be difficult to predict the outcome of such PCR-based cross-genus amplification reactions without examining a wide array of diverse *Poa* germplasm. Lastly, there were no obvious differences that would suggest that SSR primer pairs obtained from the *Lolium multiflorum* or *L. perenne* primer had a greater or lesser tendency to amplify *Poa* genomic regions.

Utilization of informative, non-SSR markers

Though only two *Poa* SSR markers were identified in this study, many of the other primer pairs generated amplification products that were polymorphic within and between the *Poa* ssp., suggesting their value as molecular markers in a marker-assisted selection program utilizing interspecific *Poa* hybrids. Several are observed to be co-expressive in a hybrid situation, suggesting co-dominant inheritance.

To illustrate the value of a non-SSR marker in genotyping and genetic analysis, the 390 bp *P. pratensis* amplification product generated by primer pair LM3–11B was applied and evaluated across a series of putative *P. arachnifera* × *P. pratensis* interspecific hybrids. In this example, the *P. pratensis* specific 390 bp amplification product is co-expressed in the inter-specific F1 hybrids and can be used to discriminate between hybrid and non-hybrid, and identify the occurrence of rare occurring androgenic haploid individuals (Figure 4) (Kindiger 2004a,b).

These types of data are valuable since the rapid and efficient identification of individual genotypes or off-type individuals following hybridization or cross-pollination can be used to increase the efficiency of individual plant selection in a *Poa* breeding program that is often slowed by the frequent generation of apomictic offspring. In addition, the utilization of such markers can reduce the need to conduct variety purity evaluations through nursery trials and phenotypic examination.

Conclusion

An early and preliminary study, utilizing a small number of *Lolium perenne* SSR primer pairs in *Poa pratensis* suggested

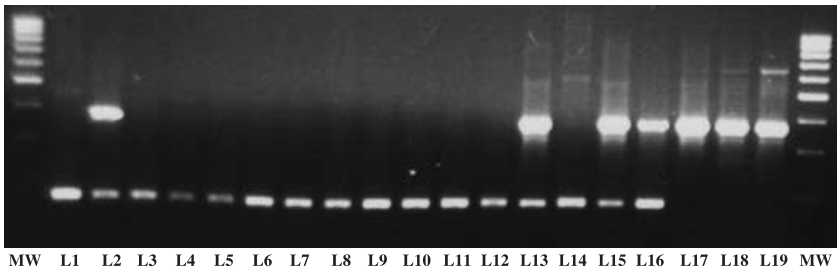


Figure 4 A genotyping example illustrating the utility of a high molecular weight 390 bp non-simple sequence repeat molecular marker identified in *P. pratensis* cv. Barrister using the primer pair LM3–11B. This band is useful to identify F1, non-F1 and *P. pratensis* androgenic haploid genotypes. L1 represents the *P. arachnifera* female parent used in the interspecific hybridizations. L2 represents a *P. arachnifera* × *P. pratensis* inter-specific F1 hybrid. L3–L12 represent non-hybrid *P. arachnifera* individuals, indicating the reproducibility and uniformity of an approximate 75 bp marker across nine *P. arachnifera* individuals. L13, L15 represent two additional F1 *P. arachnifera* × *P. pratensis* interspecific hybrids. L14 represents a non-hybrid *P. arachnifera* individual. L16 represents an equal mix of *P. arachnifera* and *P. pratensis* amplification products used as a polymerase chain reaction amplification check for identifying interspecific hybrids. L17–19 represent *P. pratensis* androgenic haploids possessing no *P. arachnifera* genome contribution. MW refers to the lanes possessing the Promega 100 bp molecular weight marker.

the successful utilization of *Lolium* SSR primer pairs to identifying complementary SSR sites in *Poa* (Kubik *et al*. 1999; Jones *et al*. 2001). The results of this study indicate that the application of *Lolium* genomic-SSR primer pairs was not widely successful in identifying complementary motif sites across an array of genetically diverse *Poa* ssp. This conclusion is supported by the poor results obtained in a similar study where SSR primers derived from a genomic library of a *L. multiflorum* × *Festuca glaucescens* F1 hybrid (Lauvergeat *et al*. 2005) were used to search for complementary microsatellite motif regions in *P. hiemata* (S.G. Byars, Centre for Environmental Stress & Adaptation Research, Melbourne, pers. comm.). Though only a single complementary *Lolium–Poa* microsatellite motif site was identified in this study, a higher degree of success may be obtained by using SSR derived from expressed sequenced tags (EST-SSR) generated from the *Lolium–Festuca* complex (Mian *et al*. 2005).

Overall, this study demonstrates that: (i) SSR primer pairs obtained from random genomic DNA of *Lolium* ssp. were relatively poor in identifying complementary SSR sites across an array of *Poa* ssp.; (ii) sequence comparisons among *Lolium* and *Poa* ssp. SSR primer amplification products suggest some level of sequence conservation as well as a lack of sequence similarity; (iii) *Lolium* SSR primer pairs can be successfully used to identify variable homoeologous loci within and among various *Poa* ssp. and; (iv) some of the *Lolium* primer pair amplification products in *Poa* ssp. can generate informative non-SSR markers that have application in a interspecific hybrid *Poa* ssp. breeding program for the detection of off-types, species introgression and validate successful inter-specific hybridization.

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